Plant, Invertebrate and Pathogen Interactions in Kosciuszko National Park

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Kosciuszko National Park is the largest protected area in NSW and the only reserve in the State containing alpine vegetation. Diseases and pests of plants in the park are poorly known and, until recently, were thought to be benign and rare because of the cold climate. Surveys after the 2003 fire that burnt about 70% of the park detected dieback in both unburnt and regenerating burnt shrubs and trees. Since then, 36 species of *Phytophthora* have been identified in the park. Some perhaps do not persist but at least two (*P. gregata* and *P. cambivora*) are affecting the survival of two native shrub species. The fungus *Armillaria luteobubalina* also has been isolated from dying shrubs. Many insects and a mite have been identified on shrubs and trees in poor health. Although some of the invertebrate and disease syndromes are likely to be cyclic and natural, their interaction with climate change and invasive species may interrupt such cycles. One threatened species, *Eucalyptus saxatilis*, is in severe decline at some sites because of insect herbivory perhaps in conjunction with unusual climatic events. Climate change is also likely to allow the invasion or expansion of non-native and native pathogens and invertebrates with unpredictable consequences.

INTRODUCTION

Kosciuszko National Park (KNP) is the largest protected area in New South Wales. Most of the Park is above 1000 m above sea level and it contains the only examples of alpine vegetation in the State (Fig. 1). Steep environmental gradients and a diversity of habitat mean that KNP has a rich flora containing many endemics (Doherty et al. 2015). The flora and vegetation of KNP face many threats. The potential impacts of climate change, introduced plants and vertebrate animals are well known (Department of Environment and Conservation 2006) but the effects of pathogens and herbivorous invertebrates (both native and introduced) have received little attention.

Perhaps the best known invertebrate herbivores in KNP are two native moth species, the alpine case moth, *Lomera caespitosae* (Lepidoptera: Psychidae) and the alpine grass grub *Oncopera alpina* (Lepidoptera: Hepialidae) that, in their larval stage, graze on the foliage and roots, respectively, of the dominant grasses in high elevation vegetation (Parida et al. 2015). Extensive patch death of grass is episodic due to outbreaks of these species but is thought to facilitate the establishment of native forbs by limiting the dominance by *Poa* species (Williams et al. 2014). Even this apparently natural process may be threatened by climate change (Parida et al. 2015).

Early reports of invertebrate damage to trees in the Kosciuszko region were associated with threats to production forestry. Phasmatids caused defoliation and death in alpine ash and mountain ash forests in the 1950s and 60s in New South Wales and Victoria (Campbell and Hadlington 1967). Outbreaks of
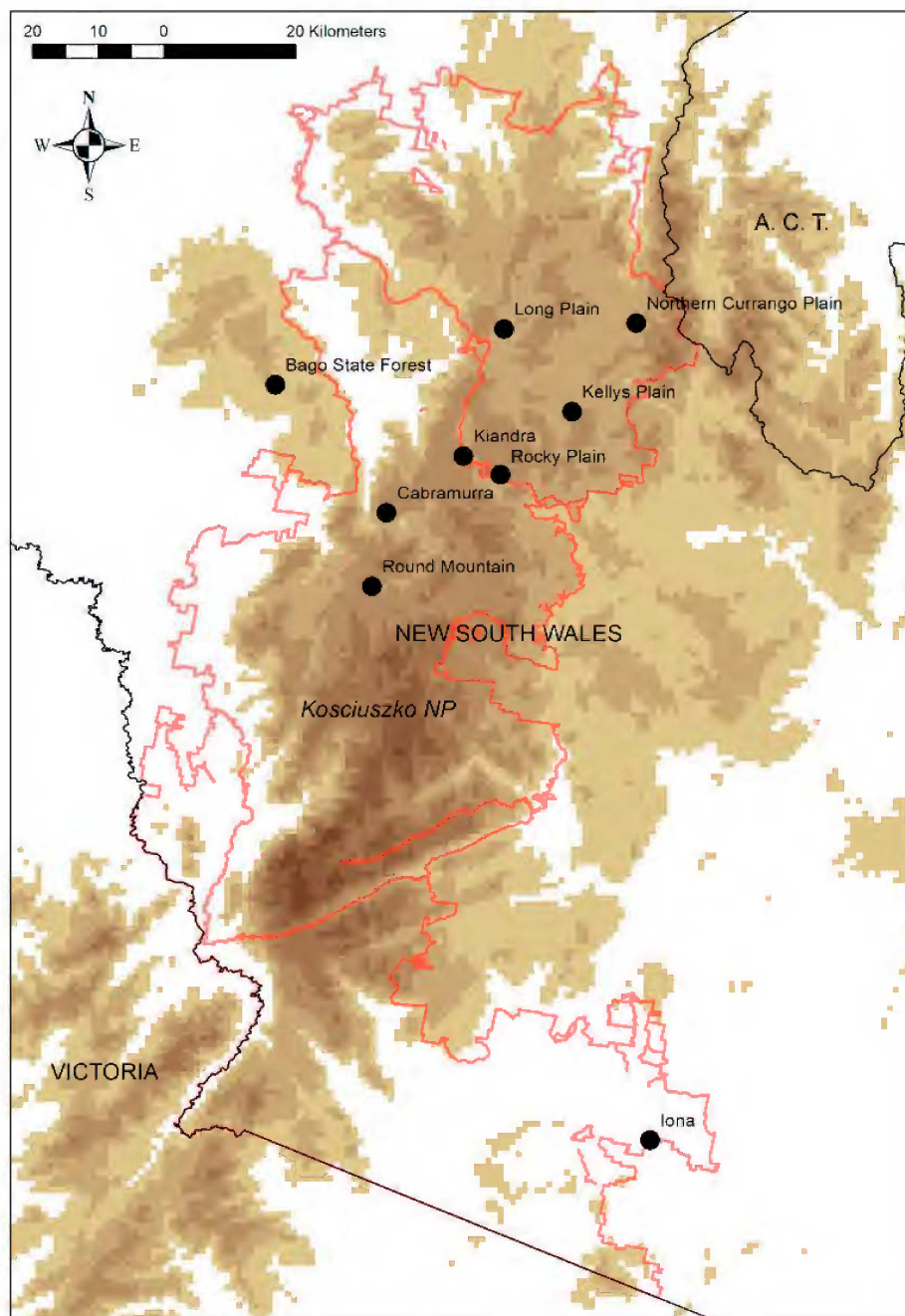
Fig. 1. Location of sites mentioned in the text. Kosciuszko National Park is shown with a red border. Elevation below 1000 m is shown in white with light to dark shades divided into 200 m elevation bands above that.

the alpine stick insect, *Didymuria violescens* are still recorded from time to time in KNP, although their effect on tree survival does not appear to be significant. The outbreaks are associated with cool, wet summers when multiplication exceeds the control exerted by predators and parasites (Readshaw 1965). In the 1980s, patch death of snow gum was recorded, especially in the vicinity of ski resorts. This was attributed at the time partly to the effects of cambial feeding by the larvae of wood moths (Lepidoptera: Cossidae: Endoxyla spp.) (Shields 1993). Again, these dieback events are likely to represent periodic natural disturbance to the native vegetation of KNP and may be associated with a range of stresses such as drought and hydrological change, that cause host trees to be more susceptible to invertebrate attack. Dieback events in eucalypts in forests and farmland have been widely studied and reported over the past 40 years and various hypotheses have been advanced regarding their cause. These include the roles of climate variation and physiological stress, outbreaks of leaf-, sap- and cambial-feeding insects and of gall-inducing arthropods and the effects of pathogens (e.g. Morrow & LaMarche 1978; Old et al. 1981; White 1986; Landsberg & Wylie 1988; Farrow 1999).

In 2013, an Australia-wide survey of *Phytophthora* species led for the first time to the testing of soils in the Alps to determine if *Phytophthora* species were present (Burgess et al. 2017). *Phytophthora* is a genus of class Oomycetes (water moulds), many species of which are pathogenic. One species, *P. cinnamomi*, has caused permanent change in numerous plant communities across southern Australia (Cahill et al. 2008). It is the only genus amongst Oomycetes and only one of three plant pathogens included in the 100 worst invasive alien plant pathogens in the Global Invasive Species Database (Lowe et al. 2000). A total of 33 *Phytophthora* species were detected through High Throughput Sequencing (HTS) using environmental DNA (eDNA) extracted from soil in KNP, about half of which are considered to be native to Australia (Burgess et al. 2017). Detection using HTS is not confirmation of a living organism as eDNA can persist in soil when the organism has died out (Carini et al. 2016). However, the isolation of *P. cambivora* from the roots of dying plants of the endemic alpine shrub *Nematolepis ovatifolia* in 2014/15 suggested the involvement of *Phytophthora* species in poor plant health (Green 2016).

Armillaria luteobubalina is a native fungus that causes root rot and wood decay of a wide range of species. Its effects on native plants and plant communities are poorly known. Although it can be readily identified from its fruiting bodies, these are not always produced. White mycelial growth on lower stems and decaying wood is often the only sign of its presence. It was first recorded in KNP in 2002 (Atlas of Living Australia; accessed 22 March 2018) but its effects on native vegetation are unknown and it is unclear whether it is native to KNP.

After wildfires that burnt about 70% of KNP in 2003, regeneration of some shrub-dominated communities was slower than expected (McDougall et al. 2015) and this was attributed in part to invertebrate herbivory and damage. This observation led to a range of studies on dieback, invertebrates and pathogens. It became clear that dieback was not restricted to regenerating burnt areas. For instance, an endangered species, *Eucalyptus saxatilis* (Suggan Buggan mallee), a small tree restricted to rocky outcrops in remote dry eucalypt forest in the Upper Snowy region of southern New South Wales and adjacent parts of northern Victoria (Brooker & Kleinig 1999), was found to be in poor health at two of its populations, yet was long unburnt. Symptoms included the loss of adult foliage, presence of dead limbs and the production of epicormic regrowth that was heavily damaged by invertebrate feeding. Numerous trees at both sites had also died.

In this paper, we present the findings of our surveys, and other observations of dieback in KNP. We conclude with management and research recommendations.

MATERIALS AND METHODS

Phytophthora species
Alps Walking Track

Targeted surveys for *Phytophthora* species along the Australian Alps Walking Track were conducted between December 2014 and April 2015. Eighty-nine soils were collected: 47 in KNP, 23 in Victoria and 19 in the ACT. At each site sample, about 300 g of soil and root material was collected beside the walking track using a trowel by scraping away the surface litter and collecting soil and roots from the rhizosphere. Sampling was done beneath plants in poor health if present. The trowel was sprayed with methanol after each sample. Samples were sent to the Royal Botanic Gardens Sydney (RBG) for the isolation and identification of *Phytophthora* species.

Pimelea bracteata

Pimelea bracteata is endemic to NSW and grows in wetlands and along waterways in northern KNP and nearby areas. Poor health in most populations of this subalpine shrub first was noticed in 2013. Possible

causes of dieback were investigated. Initial symptoms included chlorosis of lower leaves and witches broom at the stem apices, which were observed in all size classes. Repeat visits to one site (Rocky Plain) indicated that affected plants did not resprout from the base; i.e. recovery did not appear to occur once symptoms had progressed. Samples of roots of dying plants at four sites in KNP (Kiandra, northern Currango Plain, Rocky Plain and Kellys Plain) were collected in November 2016 and sent to the RBG for testing for root pathogens. After inconclusive results from these tests (with three samples testing positive for an unidentified *Phytophthora* species), symptomatic plants were examined more closely. Roots were found to be largely unaffected when the top of the plant was dead or dying. Plant collars (tissue between the roots and stems at the soil surface), on the other hand, were typically necrotic and often quite rotten. For this reason, subsequent sampling was of plant collars. Ten collars from plants at the Kellys Plain site and one from the adjoining Bago State Forest were sent to the RBG for the isolation of *Phytophthora* species.

Phytophthora detection

A dual baiting system of blue lupins (*Lupinus angustifolius*) and a *Phytophthora* selective Medium (PSM) were used to isolate *Phytophthora*. The base PSM consisted of 15% clarified V8 juice (Campbells V8 vegetable juice; Campbell Grocery products Ltd., Norfolk, UK) with a pH adjusted to 7 using 0.1 g/L CaCo3 and 2% Grade A Agar (Becton, Dickenson and Company, Sparks, MD, USA). Rifamycin, Hymexazol and Pimaricin were added to the base PSM at 10, 50 and 5 ppm, respectively. Soils and roots from each sample were mixed in a zip-lock plastic bag and flooded with de-ionized water. Pre-germinated lupins and PSM plugs were added to the soil-water slurry and incubated for 7 days at 22°C with three replicates of each baiting system per sample. Two separate methods were used for *Phytophthora* identification: microscopic examination of colony morphology from the PSM baits and total DNA extraction from the lupin radicles followed by PCR with *Phytophthora* specific primers developed by Schena et al. (2008) that targeted the ras-related protein (Ypt1) gene region. Post incubation, the PSM plugs were rinsed with de-ionized water and incubated on PSM for 3 days at 22°C for identification by gross colony morphology at 10x magnification under a light microscope. Total DNA was extracted from the distal 10mm of the lupin radicles using the FastDNA Kit (Q-biogene Inc., Irvine, California, USA) according to the manufacturer's instructions. Species identification was based on *Phytophthora* specific PCR (Schena et al. 2008) and sequencing of the nuclear ribosomal DNA, internal transcribed spacer 1 and 2 (ITS) and BLAST analysis in NCBI's GenBank. The ITS and Ypt1 were amplified using primer sets and PCR conditions described in Cooke et al. (2000) and Schena et al. (2008), respectively. Amplicons were purified using ExoSAP-IT (USB Corporation Cleveland, Ohio, USA) according to the manufacturer's instructions and sent to the Ramaciotti Centre for Gene Function Analysis at the University of NSW where DNA sequences were determined using an ABI PRISM 3700 DNA Analyser (Applied Biosystems Inc., Foster City, California, USA).

Armillaria luteobubalina

White mycelial growth was observed on a range of plant species in the vicinity of Round Mountain and Long Plain in 2014, and was suspected to be *A. luteobubalina*. The vegetation in the Round Mountain area was generally in poor health, although this may have been in part because the area had been burnt more frequently in recent times than similar vegetation elsewhere in the park. Samples of wood from the lower stems of *Grevillea australis, Phebalium squamulosum, Oxylobium ellipticum* and *Eucalyptus pauciflora* (Round Mountain) and *Eucalyptus lacrimans* (Long Plain) were sent to the RBG to test for the presence of *A. luteobubalina* (see test methods below). The subspecies of *P. squamulosum* in this area is regarded as intermediate between *P. alpinum* and *P. ozothamnoides* (Wilson 2013).

Armillaria detection

Armillaria detection was based on the multiplex PCR methods described in Guglielmo et al. (2007, 2010). Total DNA was extracted from wood and root tissues using a modified version of FastDNA Kit (Q-biogene Inc., Irvine, California, USA). The modification substituted the FastDNA Kit extraction buffer with Qiagen Inhibitex Buffer (Qiagen Inc., Germantown, Maryland, USA, Cat # 1080771) as per Guglielmo et al. (2007, 2010). Species identification was based on sequencing of the nuclear ribosomal DNA, internal transcribed spacer 1 and 2 (ITS) and BLAST in NCBI's GenBank. The multiplex PCR and ITS were amplified using primer sets and PCR conditions described in Guglielmo et al. (2007, 2010) and Gardes and Bruns (1993), respectively. Amplicons were purified using ExoSAP-IT (USB Corporation Cleveland, Ohio, USA) according to the manufacturer's instructions and sent to the Ramaciotti Centre for Gene Function Analysis at the University of NSW where DNA sequences were determined using an ABI PRISM 3700 DNA Analyser (Applied Biosystems Inc., Foster City, California, USA).

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Invertebrates

Shrub dieback

Investigations of the role of invertebrates in the dieback of four montane and subalpine shrub species were made between December 2013 and March 2014. Six sites were established among vigorous and dieback-affected communities of *Cassinia monticola, Ozothamnus cupressoides* (Kiandra area) and *Acacia obliquinervia* (Cabramurra area) to compare the invertebrate assemblages present. Plots of 100 x 10 m were randomly positioned within each site to standardise the sampling area. Observations of dieback symptoms were also made from other areas within KNP. Multiple methods were used for collecting invertebrates of various habits and behaviours (e.g. diurnal, nocturnal and flying): sweep-netting; beating trays; hand collecting; and UV light trapping. Collecting was performed at each site for one day- and night-time period during February and March 2014. Collecting by hand, sweep net, or beating tray was performed at multiple times during the day, with the UV light trap operating prior to and post sundown for approximately 2.5 hours. Evidence of invertebrate activity (e.g. feeding damage on foliage, larval and pupal case presence) was also documented. Rearing of larvae to their adult stage was performed to aid identification processes.

Invertebrate identification was performed to genus/species level where possible through use of keys, other literature sources (e.g. Common 1990; CSIRO Division of Entomology 1991) and through consultation with practised taxonomists. Voucher specimens were made available to the Australian National Insect Collection, CSIRO, Canberra, ACT. Some invertebrate groups, such as spiders (Arachnida: Araneae) and ants (Hymenoptera: Formicidae) were excluded during collecting due to their predatory habits and being unlikely to feed on plants. Life history information, obtained from available literature sources, was used to assess whether identified invertebrates were likely to cause damage to plants (e.g. through herbivory). Samples of the mites found on *Pimelea bracteata* were sent to Danuta Knihinicki (NSW Department of Primary Industries, Orange) for confirmation of the preliminary identification.

Eucalyptus saxatilis

Insect and foliage samples from *Eucalyptus saxatilis* at the Iona population in KNP north-west of Delegate were collected in December 2016 and February 2017 to identify the invertebrates responsible for the defoliation of juvenile and adult leaves.

RESULTS

***Phytophthora* species**

With the species detected by Burgess et al. (2017) and unpublished data of one author (IK), 36 *Phytophthora* species have now been recorded in KNP (Table 1).

Alps Walking Track

Of the 89 soils collected along the Australian Alps Walking Track, only one (in the ACT) was found to contain a *Phytophthora* species (*P. cryptogea*). This was in contrast to the 40 KNP soil samples tested using HTS (Burgess et al. 2017), which each contained between one and 14 *Phytophthora* species. Fifty-eight of the soil samples from the walking track contained at least one *Pythium* species, which included *P. macrosporum, P. undulatum, P. mamillatum* and undescribed taxa. *Pythium* is a genus in the same class as *Phytophthora*. Many species are pathogenic but little is known about their effect in native vegetation in Australia (e.g. Marks and Kassaby 1974).

Pimelea bracteata

Plants of *Pimelea bracteata* in poor health throughout northern KNP were found to have abnormal leaf growth at the stem apices, commonly called witches broom (in addition to the symptoms described above associated with *Phytophthora* infection). One of the authors (RF) identified minute eriophyid mites among the deformed leaves and stems and a literature search revealed that a species of eriophyid mite, *Aceria pimeliae*, induces galling on several species of *Pimelea* in New Zealand (Manson 1984). Eriophyid mite species induce witches broom galls on a wide range of plant species and are generally host specific.

Three of five initial samples taken from the roots of dying *Pimelea bracteata* at six sites in KNP tested positive for *Phytophthora* but the species could not be identified. Subsequent sampling of collars enabled the isolation of two pathogens. Of 10 samples from plants in Kellys Plain (KNP), six tested positive for *P. gregata*, and *P. cryptogea* was isolated from the single Bago State Forest plant. The collar rot symptoms were observed in plants at all sites (Fig. 2).

Armillaria luteobubalina

Armillaria luteobubalina was detected in the shrubs *Grevillea australis, Oxylobium ellipticum,* and *Phebalium squamulosum* but not in the trees *Eucalyptus pauciflora* and *E. lacrimans*.

Table 1. List of *Phytophthora* species detected in KNP through traditional baiting isolation techniques (B) and High Throughput Sequencing (S). Source: TB (Burgess et al. 2017); KG (Green 2016); IK (Ihsan Khaliq, unpublished data).

Phytophthora species	Isolation	Vegetation	Source	Elevation range (m)
P. amnicola	S	Montane forests	TB	1060 – 1320
P. arenaria	S	Montane forests	TB	740 – 1290
P. bilorbang	S	Montane forest	TB	1405
P. boodjera	S	Widespread	TB	425 – 2125
P. cactorum	B, S	Widespread	TB; IK	1340 - 1820
P. cambivora	B	Alpine heath	KG	1830
P. capensis	S	Widespread	TB	860 – 1140
P. chlamydospora	B, S	Widespread forests, subalpine woodland	TB; IK	980 - 1140
P. cinnamomi	S	Widespread	TB	455 – 2125
P. citricola complex	S	Montane forests, alpine vegetation	TB	1405 – 2065
P. cryptogea	B, S	Widespread	TB; IK	425 – 1580
P. elongata	B, S	Widespread	TB; IK	565 – 2000
P. europea complex	S	Subalpine and alpine areas	TB	1460 – 2065
P. fallax	B	Widespread	IK	425 – 1700
P. gonapodyides	B	Subalpine and alpine areas	IK	1660 - 1870
P. gregata	B, S	Subalpine wetland, montane forests	TB; IK	1280 – 1460
P. inundata	S	Montane forests, subalpine woodland	TB	1220 – 1320
P. litoralis	S	Montane forests, subalpine woodland	TB	980 – 1405
P. moyootj	S	Montane wetland	TB	1405
P. multivora	S	Widespread	TB	425 – 2065
P. nicotianae	S	Widespread	TB	860 – 2000
P. niederhauserii	S	Widespread	TB	500 – 2065
P. oreophila	B	Alpine herbfield	IK	1830
P. palmivora	S	Montane forests	TB	980 – 1220
P. parvispora	S	Montane forest	TB	980
P. pseudocryptogea	B, S	Widespread	TB; IK	425 – 1700
P. riparia	S	Montane forest	TB	980
P. rosacearum	S	Montane forest	TB	980
P. sp cyperaceae	S	Montane forest	TB	800
P. sp nov 1B	S	Montane forest	TB	1405
P. sp nov 2A	S	Montane forest	TB	740
P. sp nov 6A	S	Montane forest	TB	980
P. sp nov 9A	S	Montane forest	TB	1280
P. syringae	S	Montane forest	TB	860
P. thermophila	S	Montane forests	TB	425 – 1320
P. versiformis	S	Widespread	TB	455 - 1440

Invertebrates

Shrub dieback

Dieback-affected *Cassinia monticola* plants generally exhibited grey discoloured crowns, and appeared to be shorter with fewer flowers when compared to vigorous communities. The grey discolouration appeared to be largely due to invertebrate-induced herbivory, which exposed a "carcass" of senesced and desiccated branches. Dieback-affected *C. monticola* plants were readily observed in 2014 along the Snowy Mountains Highway, other roads near Kiandra and adjacent areas (e.g. Three Mile Dam, Gooandara Fire Trail and Racecourse Creek). The extent of dieback was variable in these areas, with *C. monticola* plants at the Gooandara Fire Trail site amongst the most severely affected. Many *C. monticola* plants persist at these sites but their health remains poor. Different types of invertebrates were observed at different sites. At Three Mile Dam and Racecourse Creek, the plants exhibited defoliation and there were dense deposits

of silk attached between the stems and branches. The silk deposits were associated with the presence of moth larvae of various stages of development. Observations of the behaviour of the larvae in field and laboratory conditions confirmed their role in causing both defoliation and depositing silk. Larvae of varying developmental stages were active at room temperature and attached dense deposits of silk to adjacent leaves, twigs and branches to form cocoons and protect them throughout pupation. Larvae were reared through development to adults, which were identified as a leaf roller *Epiphyas erysibodes* (Lepidoptera: Tortricidae: Archipini) (Ted Edwards, ANIC, CSIRO, pers. comm.) (Fig. 3). Moth larvae were also detected in low numbers within healthy patches of *C. monticola* but were not identified. Dieback-affected *C. monticola* plants at the site near Gooandara Fire Trail were found to be infested heavily with a species of leaf beetle (Coleoptera: Chrysomelidae: Eumolpinae). The beetles were observed feeding on/defoliating the plants throughout the day and initial

Fig. 2. Lesion in the collar of *Pimelea bracteata* (light brown region) with dead tissue above (to the right in the photo) and apparently healthy roots below.

Fig. 3. The larvae of the moth *Epiphyas erysibodes* (Lepidoptera: Tortricidae) on *Cassinia monticola*, showing (left) larval activity (circled in red) and dense silk attached between branches and (right) larva depositing silk threads.

counts recorded 24 beetles occupying a 1 m² area. Spot counts of the beetles within a 5 m radius of each of the 5 transect markers recorded a total of 156 individuals with numbers of the beetle reaching 56 at one location. Samples of *C. monticola* foliage and beetles were brought back to the laboratory, where the beetles were further observed to feed on and deplete available foliage. The beetle was identified as *Geloptera jugularis* (Chris Reid, Australian Museum, pers. comm.) (Fig. 4).

Dieback-affected *O. cupressoides* plants exhibited irregular patches of grey discoloured foliage (Fig. 5), black sooty mould on branches and clustering of terminal branchlets, an indication of the presence of a sugar-producing, sap-feeding, scale insects. Reduced growth and high rates of mortality (often estimated to be greater than 80% within a 5 m radius) were also observed. Invertebrate related damage of *O. cupressoides* was noted on most plants in the dieback-affected stands and rarely in healthy stands. The observed damage involved clustering of leaves at the terminal ends of branches. Silk deposits were observed on leaves but were not as dense as those observed in *C. monticola*. Observations in the field indicated that clustering of leaves was associated with soft-bodied scale insects (Hemiptera:

Coccoidea) and moth/butterfly larvae. Thirty samples of the clustered terminal branches investigated for the presence of invertebrates revealed male and female mealybugs (Coccoidea: Pseudococcidae) at various stages of development. The mealybugs were identified as belonging to the genus *Dysmicoccus* and were an undescribed species (Penny Gullan, Australian National University, pers. comm.). Attempts to recover and rear moth larvae from *O. cupressoides* foliage samples were unsuccessful. The number of species obtained from the healthy *O. cupressoides* stand was among the highest of all sites investigated. Scarab beetles (Coleoptera: Scarabaeidae) were the most abundant beetles seen on the foliage of dieback-affected *O. cupressoides.*

In a dieback-affected site near Cabramurra, very few surviving *Acacia obliquinervia* individuals were present. The dieback was characterised by extensive defoliation and an almost complete lack of branches on plants. Mortality was estimated to be greater than 95% and there were only a few leaves remaining on the surviving plants. Some individuals had presumably been dead for some time as the branches and trunk were very brittle with the entire plant easily collapsing underfoot. Observations of dieback-affected *A. obliquinervia* stands in the Thredbo region

Fig. 4. The beetle *Geloptera jugalaris* (Coleoptera: Chrysomelidae), found to be responsible for defoliation of dieback-affected *C. monticola* plants.

Fig. 5. Dieback-affected community of *Ozothamnus cupressoides* (grey foliage in the foreground with healthy plants beyond), Kiandra, Snowy Mountains Highway (February 2014).

showed extensive damage from the activities of invertebrate herbivores. Two distinct types of damage were recorded: i) defoliation caused by insects with chewing mouthparts, with characteristic scalloped edges of remaining leaves; and ii) damage of foliage caused by a leaf-skeletonising insect, resulting in rust coloured leaves when senesced.

A number of herbivorous invertebrates specific to *Acacia* were collected in surveys of the healthy *A. obliquinervia* stand. These included the leaf beetles *Calomela ioptera*, *Calomela* sp. (Coleoptera: Chrysomelidae: Chrysomelinae), the Botany Bay diamond weevil *Chrysolopus spectabilis* and other weevils (Coleoptera: Curculionidae). Galls were also commonly observed on plants with wasp larvae extracted from them in the laboratory, but their identity was not determined through rearing to the adult stage. The presence of red spider mites was recorded in both healthy and dieback-affected stands of *A. obliquinervia* near Cabramurra. However, the

assessment of dieback-affected *A. obliquinervia* conducted in this study did not reveal many invertebrates that could be considered potentially significant in plant decline nor other evidence of invertebrate damage to plants. Larvae of *Chrysolopus* feed on the roots of *Acacia* species and are reputed to destroy young trees (Hunt et al. 1996). A potential reason for the limited evidence of invertebrate related damage is that the activity at the investigated site had occurred in a previous season. A rarely encountered reticulated beetle (Coleoptera: Cupedidae) was collected in the dieback-affected site and two undescribed moth species were recorded from the surveys of healthy *A. obliquinervia*. The invertebrate responsible for skeletonisation was not determined.

The mite associated with witches broom in *Pimelea bracteata* plants (Fig. 6) was confirmed by Danuta Knihinicki (NSW Department of Primary Industries) to be *Aceria pimeleae*, previously only known from New Zealand, where it causes galling on some *Pimelea* species (Manson 1984). Curiously, witches broom was not observed on *Pimelea pauciflora*, which grows with *P. bracteata* at many sites.

Eucalyptus saxatilis dieback

Large numbers of Christmas beetles, *Anoplognathus* spp. (Coleoptera: Scarabaeidae: Rutelinae) were observed grazing on adult foliage and new leaf growth in the crowns of *Eucalyptus saxatilis* in December 2016. Unhealthy trees had produced epicormic shoots but many of these were dead at that time. The February 2017 survey found that the majority of epicormic leaves were heavily damaged by feeding by larvae of the eucalypt weevil, *Gonipterus* sp. (Fig. 7). There was also evidence of minor feeding damage by a range of other species of insect herbivore, including autumn gum moth larvae (*Mnesampela privata*), leaf tier larvae (*Tortricidae* sp.), and gumtree scale (*Eriococcus confusus*, confirmed by RF) plus a few galls induced by various species of gall-wasp (*Chalcidoidea* sp.). Many trees in poor health had been heavily colonised by a species of the plant *Cassytha melantha* (Lauraceae), a genus of hemiparasites that are reliant on their host for growth, and can have negative effects on host growth and survival (e.g. Prider et al. 2009).

Fig. 6. Witches broom (abnormal leaf growth) on *Pimelea bracteata*, Gurrangorambla Creek, KNP, caused by a mite, *Aceria pimeleae*.

a

b

c

d

Fig. 7. a) *Eucalyptus saxatilis* **with dieback at 'Iona' west ridge; b)** *Gonipterus* **sp. (Australian weevil) adult, c)** *Gonipterus* **sp. larva, d)** *Gonipterus* **sp. feeding scars on E. saxatilis leaf.**

DISCUSSION

We have identified numerous pathogens and herbivorous invertebrates in KNP in recent years. Several species appear to be associated with poor plant health but some of these are Australian endemics. It is unclear whether such species are native to KNP and, if they are, whether they are part of natural cycles. The information we have collected will form part of a baseline for future assessments of invertebrate and pathogen impacts.

Phytophthora **species**

Of the 36 *Phytophthora* species identified in KNP so far, only three have been implicated in poor plant health in and near KNP. The most serious appear to be *P. gregata* and *P. cryptogea,* which are affecting *Pimelea bracteata* populations in northern KNP and surrounding areas. Plants of all sizes of this shrub are killed and regeneration is poor or non-existent. *Phytophthora gregata* is of uncertain origin (Jung et al. 2011) while *P. cryptogea* is almost certainly non-native in Australia. Regardless of their origin, the

rapid decline in *P. bracteata* over much of its range is not consistent with a cyclical event. Interactions with climate change and the mite causing witches broom cannot be ruled out.

Only 10 of the 36 *Phytophthora* species were detected using traditional baiting of soil and root material, which promotes production of zoospores. That is, this technique identifies living *Phytophthora* species that are capable of growth and reproduction. In contrast, HTS may detect relic DNA, genetic material that is incapable of producing living organisms (Carini et al. 2016). This means that some of the 32 species detected using HTS may not persist at the sites where the samples were collected.

Phytophthora cambivora is known to infect roots of the endemic alpine shrub *Nematolepis ovatifolia* but is believed to be a contributor to decline rather than a sole cause, climatic events being of more importance (Green 2016). The origin of *P. cambivora* is also uncertain. It is a serious forestry and horticultural pathogen in many parts of Europe and North America (Erwin and Ribeiro 1996; Saavedra et al. 2007; Jung et al. 2013) and has been found to affect horticulture in Australia (e.g. Bumbieris & Wicks 1980). In spite of this, isozyme analysis of isolates from various parts of its range found most variation within Australia (Oudemans & Coffey 1991); an Australian origin for the species would therefore not be untenable.

Phytophthora cinnamomi has not been confirmed from soil / root baiting tests in KNP but it has been detected from elevations above 2000 m using HTS. If it becomes active in KNP, populations of *Xanthorrhoea glauca* could be at risk, as this species is known to be highly susceptible (McDougall and Summerell 2003). A subalpine shrub, *Phebalium squamulosum,* was found to be highly susceptible to both *P. cinnamomi* and *P. cambivora* in glasshouse pathogenicity trials (Rigg et al. 2018). Two species of *Phytophthora* detected in KNP (*P. bilorbang* and *P. cryptogea*) have been implicated in the decline of blackberry (*Rubus anglocandicans*) in Western Australia (Aghighi et al. 2015). If they also affect blackberry populations in KNP, the presence of pathogenic *Phytophthora* species in KNP need not be entirely negative for the Park's values.

Armillaria luteobubalina
Armillaria luteobubalina is a native fungus that causes plant death and contributes to the decay of dead plant material in both horticulture and natural ecosystems. In some natural ecosystems it can have a long and lasting impact (e.g. Shearer et al. 1997). It is best known for its effect on woody plants, especially trees where masses of fruiting bodies may be seen on infected trunks, but it can infect and kill plants from a wide range of families and growth forms including grasses (e.g. Shearer et al. 1998). Although native to Australia, its natural distribution is unknown. General plant health in the Round Mountain area where we detected it is unusually poor. This might be a result of frequent fire but the presence of *A. luteobubalina* there possibly hinders regeneration following defoliation and the death of stems following fire. Further survey for *A. luteobubalina* is recommended so that its distribution and possible role as a driver of vegetation change can be determined.

Invertebrates
Dieback of *Cassinia monticola* was associated with defoliation caused by infestations of an adult leaf beetle, *Geloptera jugularis* (Chrysomelidae : Eumolpinae). Polyphagous and frugivorous eumolpine beetles are recognised as having significant economic importance with genera such as *Colaspis* recorded as garden and crop pests (Jolivet & Verma 2008; Jolivet & Hawkeswood 1992). The larvae of all Eumolpinae are soil-dwelling and are root-feeders (Jolivet & Verma 2008) and can negatively affect plants through feeding at both larval and adult stages. *Geloptera jugularis* is endemic to Australia and known to be common in montane areas of Victoria and has previously been recorded on *Citrus* (Lea 1915; Jolivet & Hawkeswood 1992); however, its full host range or the extent of polyphagy in this genus is unknown. The numbers of adult beetles we recorded (up to 24 per m^{-2}) indicates that *G. jugalaris* has the potential to attain large population sizes and be destructive to *C. monticola* plants through defoliation, as observed in this study. The roles of *G. jugalaris* and other unidentified eumolpine beetles recorded by us as herbivores in KNP warrant attention in future studies.

Infestations of the leaf-rolling moth *Epiphyas erysibodes* on dieback-affected *C. monticola* plants were also identified. *Epiphyas erysibodes* is endemic to Australia and is also host specific to species in the Asteraceae family, being previously recorded from *Olearia ramulosa,* with other members of the genus recorded on other Asteraceae species (McQuillan 1992). While this study recorded only one *Epiphyas* species, there may be a suite of related species or other Tortricidae associated with *C. monticola* and other Asteraceae in KNP (Ted Edwards, pers. comm.). It was clear that this species was causing significant damage to plants in local areas and the impact of this or other related species on *C. monticola* communities in other areas of KNP requires further investigation.

An undescribed species of mealybug in the genus *Dysmicoccus* (Hemiptera: Pseudococcidae) also was found associated with the clustered branchlets of dieback-affected *O. cupressoides*. There have been no previous records of these mealybugs on *Ozothamnus* and the closest species morphologically to *Dysmicoccus* sp. found in this study is *D. banksi*, occurring on various *Acacia* spp. (Penny Gullan, pers. comm.). Mealybugs are sap-sucking insects and damage plants directly by inserting their mouthparts and sometimes also toxic compounds into plant tissue when feeding. Their populations can build rapidly due to high reproductive capacities and multiple generations per year and are often serious pests of plants. The waste product of sap-sucking insects ("honeydew") may further compromise plant health by providing suitable growing conditions for sooty mould fungi. The black sooty deposits observed on dieback-affected *O. cupressoides* plants may be a result of this type of feeding interaction between *Dysmicoccus* spp. or other sap-sucking Hemipterans such as aphids, scale or coccids. Further study is needed to determine whether this condition contributes to dieback in *O. cupressoides* plants.

Chafer beetles (Coleoptera: Scarabaeidae: Melalonthinae) were recorded in surveys from all sites, with the highest number of species obtained from dieback-affected *C. monticola* and *O. cupressoides*. These beetles may be significant as they feed on a wide variety of plants, the adults commonly swarm and defoliate plants en masse, and the root-feeding larvae can occur in very high densities. However, identification was not possible in the current study because of the diversity of the group and the large number of undescribed species.

Invertebrates collected in surveys of *A. obliquinervia* that may have contributed to dieback were the leaf beetles (Coleoptera: Chrysomelidae: Chrysomelinae) and weevils (Coleoptera: Curculionidae). Two species of Acacia leaf beetles from the genus *Calomela* were obtained from the healthy stands of *A. obliquinervia*, with *C. ioptera* being previously recorded from this plant host (Reid 1989). *Calomela* beetles are known to specialise in feeding on *Acacia* with some species known for extensive defoliation of *Acacia mearnsii* (Selman 1979; Hunt et al. 1996; Reid 2006). The Botany Bay diamond weevil *Chrysolopus spectabilis* was also recorded in the survey from the healthy stand of *A. obliquinervia*. Adults and larvae are common on a wide range of *Acacia* species in plantations and natural stands and larvae are alleged to cause the death of young trees (Hunt et al. 1996). Other weevils

were also recorded in the survey, but their potential to affect local *Acacia* species is unknown.

The dieback symptoms observed in *A. obliquinervia* were similar in appearance to that caused by outbreaks of the fireblight beetle, *Peltoschema orphana* (Chrysomelidae: Chrysomelinae) on *Acacia mearnsii*. The name given to this beetle refers to the scorched brown appearance of stands of attacked *Acacia*. Outbreaks of fireblight beetle commonly occur in black and silver wattle plantations, and in forests containing large natural stands of these species (Elliott 1978; Elliott et al. 1998). Repeated seasonal defoliation through feeding by *P. orphana* causes heavy mortality to these acacias. There is also anecdotal evidence that outbreaks of *P. orphana* occur in regenerating host acacias after fire events. It was not possible to determine whether leaf beetle species found in this study were directly responsible for the extensive defoliation of *A. obliquinervia* plants as only photographic evidence of activity after feeding events was available. Nevertheless, a number of leaf beetles known to cause extensive defoliation of host plants are present in KNP (e.g. *Calomela* and *Geloptera* spp.), suggesting that future studies on their impact could focus on adult and larval activity in relation to known patterns of seasonal abundance.

Eucalyptus saxatilis is facing pressure from a large number of insect species and a parasitic vine. The species of *Gonipterus* that is consuming its epicormic shoots is also reported to be responsible for defoliation of *Eucalyptus viminalis* in nearby areas of the Monaro leading to the widespread death of host trees (Ross and Brack 2015). These weevils are particularly attracted to epicormic regrowth of juvenile foliage and there was little evidence in *E. saxatilis* that the larvae were feeding on adult foliage. Christmas beetles (*Anoplognathus* spp.) on the other hand were found to be feeding abundantly on new and mature foliage in early summer, and other insect herbivores were recorded on foliage. Outbreaks of insect herbivores are often a consequence of abiotic stresses such as drought (e.g. see the review of Anderegg et al. 2015). Many factors are probably contributing to decline in *E. saxatilis* and a changing climate may further stress plants and facilitate greater pressure from insect herbivores. Monitoring of plant health and insect populations is recommended so that causes and an appropriate management response can be identified. As a precursor to that, a small number of dieback-affected trees were injected with the insecticide imidacloprid in October 2017. The aim of this trial is to investigate whether relieving browsing pressure will allow dieback-affected trees to recover.

Fig. 8. Subalpine vegetation at Kellys Plain, KNP, before (left, December 2015) and after (right, January 2017) extensive death of Poa species caused by moth larvae. The non-native, white-flowered daisy *Leucanthemum vulgare* (ox-eye daisy) colonised the dying grasses more rapidly and in much greater densities than the native forbs that are usually favoured by this disturbance.

Interactions and the future

Although some of the effects of the pathogens and invertebrate herbivores that we recorded may be naturally cyclic, the cycles may be affected by climate change, which could facilitate the introduction of new pathogens and invertebrates or make native plant species more vulnerable to their effects. In addition, invertebrate and pathogen ranges are likely to shift more rapidly than their plant hosts, which will experience movement lags for a variety of reasons (Alexander et al. 2018). This may result in host shifts or changes in the frequency of outbreaks. For instance, in the European Alps, the optimal climatic niche of the larch budmoth, *Zeiraphera diniana* (Lepidoptera: Tortricidae) has shifted in recent decades causing a large reduction in outbreaks (Johnson et al. 2010). Pathogens may also exert pressure beyond their host and lead to cascading effects. In the Rocky Mountains of North America, an exotic pathogen, *Cronartium ribicola,* is causing poor health and death in the treeline species *Pinus albicaulis*, a keystone species that is an important food source for native vertebrates and a facilitator for the growth of other plant species (Tomback and Resler 2007). Widespread death of

P. albicaulis will therefore have flow on effects and possibly confound expected treeline movement upslope. An increase in the frequency of outbreaks of insect herbivores (e.g. moth larvae (Shields 1993)) feeding on the KNP treeline species *Eucalyptus niphophila* associated with increased climatic stress (e.g. from more frequent drought) could also have cascading effects, including hydrological change (e.g. Costin and Wimbush 1961), and changes to snow accumulation and persistence (Costin et al. 1961).

Natural invertebrate – plant cycles may also be affected by non-native plant introductions. Natural episodic death of *Poa* species in treeless vegetation throughout the Alps associated with two moth species appears to have been hijacked by the invasion of the non-native vascular plant (*Leucanthemum vulgare*), which has invaded large areas of grassland in northern KNP. This daisy grows much faster than most native forbs, regularly produces large quantities of seed and readily colonises bare ground. Its density was found to increase rapidly after extensive grass death from moth larvae (Fig. 8) and its dominance may be leading to further decline in *Poa* cover (McDougall et al. 2018). A reduction in *Poa* cover in grasslands could mean

that future outbreaks of tussock death from native moth larvae will be more restricted and colonisation opportunities for native forbs reduced. In addition, one of the moth species may not be favoured under future climate (Parida et al. 2015).

Because of steep climatic gradients in the Alps, small increases in temperature could lead to large elevational shifts of suitable climatic niche for native and non-native pathogens and invertebrates. For instance, an analysis by Podger et al. (1990) in Tasmania found that disease caused by *P. cinnamomi* is unlikely to occur where mean annual temperature is below 7.5° C and annual mean rainfall is less than 600 mm. In much of KNP, this corresponds to an elevation of about 1300 m, which may explain why persistent populations of *P. cinnamomi* have yet to be detected. However, by 2070, the 7.5°C isotherm may be up to 500 m higher under an intermediate emissions scenario, encompassing all populations of *Phebalium squamulosum*, a species which is especially susceptible to *P. cinnamomi* (Rigg et al. unpublished data). At the same time, the optimal climate for the *Phebalium* is likely to shift upwards. However, its capacity for dispersal is much poorer than *P. cinnamomi* and a demise from disease is plausible. Non-native invertebrates are being recorded in the Australian Alps largely as a result of niche filling (Nash 2013), but a changing climate will allow invasion of a large range of new species. One species that has invaded, the European honey bee (*Apia mellifera*), has reached the alpine area in both Victoria and KNP (Inouye and Pyke 1988, Nash 2013). Changes in pollinator composition could affect plant reproduction in unpredictable ways (Nash 2013). The effects of invertebrates on plants in KNP therefore may not be restricted to herbivory.

The climatic niche of many mountain plant species is expected to contract or disappear in the future under current climate projections. If that occurs, some of the pathogens and herbivorous invertebrates that rely on them may themselves become rare and threatened.

Management and research needs

It will be difficult to limit the spread of soil-borne pathogens along the network of public roads in KNP. Minimising the movement of soil-borne micro-organisms through conventional hygiene measures (e.g. foot scrubbing stations, vehicle wash down, signage, education) is still valuable, as it will also limit the movement of non-native seed. Woodchips produced from areas found to be infested with Armillaria should not be used in other areas for revegetation to prevent its spread. A reduction in the vectors likely to spread pathogens (e.g. pigs, horses, deer) is also wise. Minimising the spread of organisms such as *Phytophthora cinnamomi* to prevent occupation of their full climatic niche will buy time to protect species at risk (e.g. through seedbanking or identification of refugia). *Pimelea bracteata* and *Phebalium squamulosum*, both susceptible to *Phytophthora* species, should be targeted for such actions in the near future.

A severe impediment to managing pathogens and invertebrate herbivores in KNP is the lack of baseline data both in terms of what is native to the park and where they are likely to occur. Such information is rarely available but it is essential for managing change and, in particular, identifying the difference between natural cycles and irreversible change. Where so little is known about threats from pathogens and invertebrates, we recommend a precautionary approach where species are managed by the impact observed rather than whether pathogens and invertebrates are native or introduced. Our studies will provide valuable data for future assessments of poor plant health. Monitoring of plant health, susceptible species populations, pathogen distribution and herbivorous invertebrate populations will assist in identifying when management intervention is required.

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